Central obesity and not age increases skeletal muscle lipids, without
influencing lean body mass and strength

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Abstract

**Background/Aims:** To measure skeletal muscle lipid infiltration, its association with insulin resistance (IR) lean mass and function, in Chilean men differing in age and body composition. Our hypothesis was that muscle lipid accumulation would be higher among older and heavier individuals and this would deteriorate insulin sensitivity (IS) and decrease muscle mass and function, both features of the ageing process.

**Methods:** Healthy men (38 < 55 and 18 > 65 years), underwent anthropometric measurements, body composition assessment through radiologic densitometry, Nuclear Magnetic Resonance spectroscopy at the tibia and quadriceps, 12 minute walking distance and serum biochemistry (haemoglobin, lipoproteins, creatinine, ultra-sensitive C Reactive Protein, fasting and post glucose insulin and glucose concentrations, to assess IS). Physical activity was estimated by actigraphy.

**Results:** 23 men were eutrophic, 26 were overweight and 7 were obese and mostly sedentary, independent of age. Both IMCL and EMCL were higher in overweight/obese men. Abdominal fat was negatively associated with IS and positively correlated with muscle lipid accretion (both IMCL and EMCL), but not with age. As expected, older individuals had lower muscle mass and strength, but not more adipose tissue nor intramyocellular lipids, yet were more glucose intolerant.

**Conclusions:** central obesity was associated with IMCL and EMCL infiltration and IR. This type of lipid accretion was not related with ageing nor age-related sarcopenia. Older individuals were more glucose intolerant, which was explained by a decrease of insulin secretion more than adiposity-related IR.

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Abreviations

IR = Insulin Resistance.
BMI = Body mass index.
IS = Insulin Sensitivity.
DREA = Double beam densitometry.
IMCL = Intramyocellular lipids.
NMR = Nuclear magnetic resonance.
EMCL = Extramyocellular lipids.
TA = Tibialis anterioris.
TAG = Triacylglycerol.
AEE = Average energy expenditure.
DAG = Diacylglycerol.
MEEE = Maximal exercise energy expenditure.

Introduction

Ageing induces well known changes in body composition, characterized by progressive building of fat mass and decline of skeletal muscle, which becomes gradually infiltrated with lipids1,2. Other age-induced changes of muscle tissue are accumulation reactive oxygen species (ROS) and byproducts of oxidative damage3,4, altered mitochondrial function5, loss and shift towards type-II fibers6, telomere shortening7 and increased expression of inflammatory cytokines8,9. At a functional level, ageing is manifested by a decline of muscle strength and endurance10. This tissue is also critical for glucose homeostasis, accounting for up to 80 % of insulin mediated, glucose uptake11. The relationship between central obesity and insulin resistance (IR) is well known but, more than total body fat mass, it has been proposed that muscle tissue accretion of triacylglycerol (TAG) and lipid intermediates are responsible for altering IS12,13. The underlying hypothesis states that when IR is no longer able to protect from lipid overload, lipids are accumulated ectopically in muscle, abdominal visceral and hepatic fat depots, along with macrophage infiltration and inflammation14, leading to further IR15 and oxidative stress. However, exercise improves insulin sensitivity (IS) together with an increase of intramyocellular lipid (IMCL) content16, a phenomenon called the training paradox, because insulin resistant, untrained obese and diabetic patients exhibit an IMCL accumulation comparable to that of the most insulin sensitive, lean and trained individuals17. Consequently it has been postulated that, apart from TAG, certain metabolites (specifically ceramide (CER) and diacylglycerol (DAG)), might be responsible for altering IS18-19. Moreover, deterioration of muscle quality by lipid infiltration is related with sarcopenia among elderly and diabetics20-21. Muscle lipid deposition assessed through nuclear magnetic resonance (NMR) spectroscopy, allows to precisely assess whether lipids gather inside muscle cells (IMCL) or surrounding them, as extramyocellular lipids (EMCL). The predominance of EMCL over IMCL depends on body composition, gender, ethnicity and endocrine environment. In tissue samples, only electron microscopy permits to assess localization of lipids in muscle cytosol (IMCL) or in adipocytes located between fibers (EMCL) or its proximity to mitochondria. Computed tomography technology identifies attenuation in the muscle area as an indicator of lipid infiltration, but is considered less accurate in defining its exact localization, due to inconsistency in cutoff Hounsfield units22.

Most studies employing NMR have been performed in subjects from American-European or African American ethnic origin in soleus and/or tibialis anterioris muscles and have detected inverse correlations between insulin sensitivity (IS) and intramuscular lipids, predominantly among subjects from European origin23. African Americans are more insulin resistant and prone to diabetes in spite of lower visceral and hepatic fat depots. Also, at comparable IMCL muscle lipids are neither correlated with visceral fat nor with IS among African Americans, while these associations are evident among European Americans. None of the mentioned studies report relation with muscle function and customary physical activity.

Because of discrepancies between studies concerning the pathophysiologic relationship between IR, muscle function and lipid accretion in muscle, it is important to add information, and also include other ethnic groups. Our hypothesis was that skeletal muscle lipid accumulation would be higher both among heavier and older individuals, in association with a decline of lean mass and muscle function (strength and walking capacity) and decrease of IS, and that overweight/obese men would behave similar to elderly. Thus the aims of the present study were first to investigate factors associated with muscle lipid accumulation (age, body composition, fat distribution, metabolic parameters), and second the possible metabolic and functional consequences of muscle deposition of lipids and its localization within the tissue (IMCL or EMCL), in healthy adult and elderly men from Latin America, where scarce information has been gathered. In trying to study relatively healthy individuals we excluded those with extreme body weights, and included only men because we have detected better associations between lean mass and muscle function in this gender24.

Methods

After signing an informed consent, we selected healthy adult male subjects, below 55 years (YA) or over 65 years (OA). Other requisites were moderate alcohol intake (less than 30 g/day), smoking less than 5 cigarettes/day, performing less than 5 hours/week of physical activity, body mass index (BMI) > 20 and < 35 kg/m², and absence of chronic diseases or intake of steroids. The study followed guidelines for human stu-
Volunteers were instructed to refrain from performing physical activity the day before the study. Each subject underwent the following assessments:

1) Anthropometry: weight and height for calculation of body mass index (BMI), waist and hip circumferences.

2) 1H Magnetic Resonance (NMR) Spectroscopy at the Radiology Unit of Clínica Alemana de Santiago, in the tibialis anterior (TA) muscle of the right calf using the STEAM (Stimulated Echo Acquisition Mode) technique, with water suppression on a GE 1.5 Signa HDxt MR unit. Measurements were acquired with the volunteer lying in the supine position with right leg positioned inside a commercially made radiofrequency extremity coil with knee configuration, with the knee in extension and ankle in a neutral position. The voxel box was placed in the most prominent muscle area, with prior identification of no lipid stranding adjacent or inside the planned zone. Peak positions and areas of interest were determined by time domain fitting using Java-based magnetic resonance user interface (SAGE29). Peak positions of IMCL and EMCL lipids were recorded, as also area under the curve (AUC) for a fixed region between 1 and 2 ppm. Results were expressed as arbitrary units (AU), reflecting the height of the peaks for extra and intra myocellular lipids. Results were not corrected by the creatine peak, since this can lead to errors when calf muscles are assessed23.

3) Body composition measurement by DEXA with Lunar Encore equipment. Central abdominal fat was calculated according to Carey31; as a better indicator of visceral fat, comparable to that measured by MRI.

4) Fasting blood samples were obtained for blood chemistry (hemoglobin, lipoproteins, us C Reactive Protein and creatinine). Three fasting samples were taken prior to the glucose challenge and then blood samples were taken at 30, 60, 90 and 120 min following the glucose challenge to determine insulin sensitivity using both the Homeostasis Model Assessment (HOMA-IR)23 and Matsuda formula28, and estimate insulin secretion through the corrected insulin response 30 minutes post glucose ingestion27. Samples were immediately transported to Vida Integra Lab, Santiago Chile where biochemistry and insulin levels were measured by automated methods the same day.

5) As indicators of muscle function, we measured hand grip strength using a handgrip dynamometer, isometric quadriceps strength in a quadriceps table and twelve-minute walk, as previously described28.

6) Placement of an Actiheart® actigraph, to record heart rate and movements during 24 hours. Activity energy expenditure was calculated through the software of the equipment, which uses a previously reported branched model algorithm29. Results were expressed as average energy expenditure (AEE) and maximal exercise energy expenditure (MEEE), in METS (Metabolic Equivalent of Task).

Sample sized was estimated based on comparison of tibialis anterior IMCL between normal weight and obese women30, since data concerning the effects of age on IMCL are less conclusive. All statistical analyses were performed in STATA 12.0. Groups were compared through Students T test, ANOVA or Kruskall Wallis according to number of groups and normality of its distribution. Associations were analyzed using Pearson or Spearman correlation coefficients depending on distribution of the variables. We also performed a multiple linear regression analysis, using 120 min. serum glucose as the dependent variable and those significantly associated on univariate analysis as independent variables. In addition, to assess factors associated with muscle strength decline, we performed a multiple regression analysis using quadriceps muscle strength as the dependent variable and age, lean body mass, central body fat and intramyocellular lipids as independent variables. Data are presented as mean ± SD for normal distribution, or as median (range) otherwise.

Results

We included 56 men, 38 YA and 18 OA, representative of Santiago’s genetic blend (mostly European-Amerindian)31. Among YA, 15 were normal weight (BMI < 25 kg/m²), 18 overweight (BMI > 25 and < 30 kg/m²) and 5 obese (BMI > 30 kg/m²). Using the same cutoff values among OA, 8 were normal weight, 8 overweight and 2 obese. Maximal BMI reached 33.8 kg/m².

Table I depicts comparison of anthropometry, body composition, metabolic parameters and muscle tissue lipids and function between both age groups. It is noteworthy that, although OA had less lean body mass, muscle strength and endurance than YA, no differences were observed between age groups in total, trunk nor central abdominal fat, intra and extramyocellular lipids and insulin sensitivity, measured through HO-MA-IR or Matsuda index. Only a weak correlation was detected between EMCL and age (Table III). However OA were more glucose intolerant, suggesting a predominant defect in β cell insulin secretion, as calculated by the corrected insulin response at 30 min (0.005±0.001-0.037) in YA versus 0.004 [0.001-0.011] in OA, p = 0.03. Serum glucose levels 120 min post glucose load correlated with age, body fat and EMCL,
but not with IMCL. The linear multiple regression analysis, using 120 min serum glucose as the dependent variable and age, abdominal fat, EMCL and 120 min insulin levels as independent variables, showed that only age and post glucose insulin levels remained as significant factors.

When dividing the total sample according to BMI categories (below or over 25 kg/m²), IMCL and EMCL were significantly higher in overweight compared to normal weight subjects (IMCL = 0.11 [0.03-0.28] versus 0.167 [0.07 – 0.45] AU, p = 0.034 and EMCL= 0.31 [0.08 - 0.64] versus 0.56 [0.03 - 2.3] AU p= 0.027). There was a significant correlation between abdominal and trunk fat accumulation with IMCL and EMCL (Table II). Insulin sensitivity assessed through HOMA-IR and Matsuda correlated significantly with IMCL but not with EMCL. Insulin secretion was negatively associated with EMCL (rho = - 0.38, p= 0.005). We found no association between total fat, abdominal fat nor muscle lipids with subclinical inflammation assessed by C reactive Protein serum levels. Muscle strength was associated with age, lean body mass and IMCL but no correlations were found with EMCL and abdominal fat. However, only age and lean body mass remained as significant predictors of muscle strength in the multiple regression analysis performed.

Twenty-three volunteers (12 YA and 11 OA) were classified as glucose intolerant, defined as a fasting serum glucose > 100 mg/dL or 2-hour glucose > 140 and ≤ 200 mg/dL. HDL cholesterol was higher among old glucose tolerant subjects and triacylglycerol levels were higher among glucose intolerant participants.

### Table I

<table>
<thead>
<tr>
<th>Distribution of study variables according to age groups</th>
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<tr>
<td><strong>YOUNGER ADULTS (YA)</strong> &lt;br&gt; (n = 38)</td>
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<tr>
<td>AGE (years)</td>
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<tr>
<td>BODY MASS INDEX (kg/m²)</td>
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<td>WAIST CIRCUMFERENCE (cm)</td>
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<td>WAIST/ HIP RATIO</td>
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<td><strong>TOTAL BODY FAT (kg)</strong></td>
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<td><strong>TOTAL LEAN BODY MASS (kg)</strong></td>
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<td><strong>TRUNK FAT (kg)</strong></td>
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<td><strong>CENTRAL ABDOMINAL FAT (kg)</strong></td>
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<tr>
<td><strong>CENTRAL ABDOMINAL FAT (%)</strong></td>
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<tr>
<td>TOTAL CHOLESTEROL (mg/dL)</td>
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<tr>
<td>HDL CHOLESTEROL (mg/dL)</td>
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<td>TRYGLICERYDES (mg/dL)</td>
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<td>FASTING GLUCOSE (mg/dL)</td>
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<td>SERUM CREATININE (g/dL)</td>
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<tr>
<td>C REACTIVE PROTEIN (mg/L)</td>
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<tr>
<td>SERUM GLUCOSE 120 MIN (POST 75g GLUCOSE LOAD) (mg/dL)</td>
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<tr>
<td>FASTING INSULIN (U/dL)</td>
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<tr>
<td>MATSUDA</td>
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<td><strong>HANDGRIP STRENGTH (kg)</strong></td>
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<td><strong>QUADRICEPS STRENGTH (kg)</strong></td>
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<td>12 MIN WALK (min)</td>
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<td>INTRAMYOCYTOCELLULAR LIPIDS (IMCL) (AU)</td>
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<tr>
<td><strong>INTRAMYOCYTOCELLULAR LIPIDS (EMCL) (AU)</strong></td>
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*Total body fat, total lean body mass and trunk fat assessed automatically by DEXA,
*Central abdominal fat calculated manually from DEXA, according to Carey et.al. [21]
*Mean values between muscle strength in right and left extremities.
young volunteers. No differences in other parameters between glucose tolerant and intolerant participants were observed within age groups (Table III).

According to actigraphic readings, both groups of men were mostly sedentary; mean activity energy expenditure was similarly low among OA (0.21 [0.1 – 0.3] METS/day) and YA (0.35 [0.1 - 0.9]) METS/day (p = 0.24); likewise, maximal exercise energy expenditure was not different (5.4 [2.9–10.5] in OA versus 4.9 [2.9–14.6]) METS/day in YA) (p = 0.60). No association was found between actigraphically estimated energy expenditure and muscle lipids.

Discussion

In this study performed in healthy Hispanic men with weights ranging from normal to mild obesity, we confirmed that intramyocellular lipid accretion increases at higher body weight, especially when fat accrues at the trunk, but did not confirm an association with ageing as found by Crane et al, using electron microscopy51 or Nakagawa et al through 1H-MRS39. Noteworthy, our elder individuals were more glucose intolerant but not heavier than young volunteers, suggesting that the predominating mechanism is age-related β cell failure and probably not peripheral IR linked to abdominal obesity.

In our sample we found that abdominal and skeletal muscle lipid accretion coincided with IR and glucose intolerance as expected, but fat accretion was not associated with sarcopenia (age related decrease of lean body mass and strength), as occurs during the ageing process. Indeed our data show that these older but healthy men had less lean mass and muscle strength as expected, but this was not associated with growth of abdominal fat and accumulation of lipids at the tibialis anterioris (TA) muscle. Inversely, in the younger individuals, IR was associated with abdominal fat and muscle lipid deposit, but the latter did not alter muscle mass and function.

Based on these data we can suggest that muscle lipid infiltration at the TA is not a consequence of ageing, because it ensued in younger men with central obesity and IR. It is possible that muscles of these YA with central obesity in the future will deteriorate more than those from the elders included in this study, because our OA group represents the healthy survivors that lived their youth in a different nutritional environment, where the metabolic syndrome was less prevalent. However the answer to this would require long-term follow up studies.

Previous investigations attributed IR34 predominantly to accumulation of IMCL, and more specifically lipid toxic moieties such as CER and DAG53, but most of these studies have been performed in heavier subjects from different ethnic origin, and IR has been assessed by hyperinsulinemic clamps6,37. Actually in these same volunteers we found significantly higher concentrations of certain CER and DAG species in biopsies obtained from the abdominal oblique muscle among men with abdominal obesity and glucose intolerance, independent of age38. However, not all studies coincide in considering IMCL, as indicator of lipid overflow that cannot be sustained within adipose tissue as the cause of IR and glucose intolerance. As mentioned, some discrepancies can depend on characteristics of the studied population (age, nutritional status, fitness, ethnic background, etc), the muscle analyzed (soleus or tibialis anterioris) and the experimental model employed (infusion of lipid emulsions, cross-sectional analysis of muscle lipid content or analysis of IMCL after caloric restriction or exercise)39,42. Our results are in accordance with those that highlight the relevance of lipid depots located within (IMCL and EMCL) and not between muscle fibers; the latter is another fat depot located under the muscle fascia, which tends to increase with weight gain and ageing, especially among African Americans41, but has not been studied among Hispanics. We are aware of only one study including Latin Americans, describing increased IMCL and hepatic lipid accretion among obese Hispanic adolescents42.

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We must point out that even though our subjects were only slightly overweight on average, their trunk adiposity was significantly higher compared to other studies, performed in elderly subjects, nonetheless were more insulin sensitive\(^43\), highlighting the relevance of confounding factors such as ethnicity, age, physical activity and body composition, when comparing results and drawing conclusions.

Apart from IR and sarcopenia\(^44\) myosteatosis has also been linked with cancer cachexia\(^45,46\), and can be modified by resistance training\(^47\). However evidences to support this premise are based on histologic analysis.
of muscle tissue in animals, or muscle attenuation by computed tomography, but have not been confirmed by NMR spectroscopy. This is the first study to discard the association between muscle fat accumulation and sarcopenia.

In this study we were especially careful in the selection criteria, in order to avoid confounding factors. The sample size was comparable to other published studies and we chose only men with similar body weights and physical activity levels which was assessed by actigraphy, a reliable methodology, to evade the training effect on muscle lipid deposition. And even though we employed the best available techniques for assessment of body composition and lipid deposition within muscle tissue, our study has several limitations. First of all, it intended to study the relevance of muscle lipid accumulation through a cross sectional analysis, but it is impossible to precisely control all other possibly influencing factors, such as dietary habits and previous physical activity, since information obtained from dietary recalls or physical activity questionnaires are not reliable enough. We ensured though that volunteers were mostly sedentary through actigraphic readings, performed during 2 days and after obtaining blood samples and performing MRIs. Other approaches have evaluated the changes in these variables after interventions that reduce muscle lipid deposition through energy restriction, exercise, or drugs, and so are not comparable. Additionally, for better NMR acquisition, muscular lipids were measured at the tibialis anterioris muscle. Whether IMCL in soleus (a predominantly oxidative muscle) is more related with IS compared with tibialis anterioris is a matter of debate, and probably depends on multiple factors, as previously mentioned. In relation to quadriceps strength and walking capacity, it must be mentioned that accurate NMR peaks (intra and extramyocellular) cannot be adequately identified in this muscle (CS personal communication), and thus attenuation (indicating lipid accretion) in rectus femoralis is usually assessed only through CT scans; however even though this method is less precise, it could potentially show better associations with this muscle’s function.

In summary, in healthy moderately active or sedentary men, we found an association between abdominal lipid accretion and TA lipid infiltration, predominantly inside muscle fibers (IMCL), in concomitance with IR. Muscle lipid infiltration starts at young ages, but it is not associated with the deterioration of muscle mass and strength observed among elderly men.

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All authors declare no conflict of interest.

References


